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 1. Document ID: US 5543312 A

Entry 1 of 2

File: USPT

Aug 6, 1996

DOCUMENT-IDENTIFIER: US 5543312 A

TITLE: *Pastuerella haemolytica* glycoprotease gene and the purified enzyme

BSPR:

P. haemolytica is the principal microorganism associated with bovine pneumonic pasteurellosis, a major cause of sickness and death in feedlot cattle in North America. Martin et al. 1980. "Factors associated with mortality in feedlot cattle: The Bruce County beef cattle project." Can.J.Comp.Med.; Yates, W. D. G. 1982. "A review of infectious bovine rhinotracheitis, shipping fever pneumonia and viral-bacterial synergism in respiratory disease of cattle." Can.J.Comp.Med. *P. haemolytica* has been divided into sixteen serotypes base on soluble or extractable surface antigens. Biberstein, E. L. 1978. "Biotyping and serotyping of Pasteurella haemolytica." Methods Microbiol. Among the sixteen serotypes, serotype A1 is the predominant microorganism isolated from pneumonic lungs. Smith, P. C. 1983. "Prevalence of Pasteurella haemolytica in transported calves." Am. J. Vet. Res.; Yates, W. D. G. 1982. "A review of infectious bovine rhinotracheitis, shipping fever pneumonia and vital-bacterial synergism in respiratory disease of cattle." Can. J. Comp. Med. *P. haemolytica* A1 produces a number of antigens which are secreted into the culture supernatant during its growth. These antigens include a heat-labile cytotoxin specific for ruminant leukocytes, Shewen et al. 1988. "Vaccination of calves with leukotoxic culture supernatant form Pasteurella haemolytica." Can. J. Vet. Med., a serotype-specific outer-membrane protein, Gonzalez et al. 1986. "Cloning of Serotype-Specific Antigen form Pasteurella haemolytica A1." Infect. Immun., a glycoprotease specific for sialoglycoproteins, Otulakowski et al. 1983. "Proteolysis of Sialoglycoprotein by Pasteurella haemolytica Cytotoxic Culture Supernatant." Infect. Immun. and neuraminidase Frank, G. H. 198. "Neuraminidase Activity of Pasteurella haemolytica Isolates." Infect. Immun. Vaccination of calves with bacterial-free culture supernatant form logarithmic phase cultures induces resistance to experimental challenge. and a vaccine based on the culture supernatant has been developed (Presponse.TM.) Shewen et al. 1988. "Efficacy testing a Pasteurella haemolytica extract vaccine." Vet.Med.; Shewen et al. 1988. "Vaccination of calves with leucotoxic culture supernatant from Pasteurella haemolytica." Can.J.Vet.Med.

BSPR:

Culture supernatants from a majority of the serotypes of *P. haemolytica* A1 contain a neutral protease (Otulakowski, G. L. P. E. Shewen, A. E. Udo, A. Mellors, and B. N. Wilkie. 1983. Proteolysis of Sialoglycoprotein by Pasteurella Haemolytica Cytotoxin Culture Supernatant. Infect. Immun. 42:64-70. FIG. 9 (A) shows that the culture supernatant from *P. haemolytica* A1 biotype A, serotype 1, contains an enzyme activity that cleaves human [¹²⁵I]-labelled glycophorin A, as revealed by the disappearance of dimeric glycophorin A and monomeric glycophorin A (lane a), to yield corresponding dimeric and monomeric products (lane b). Glycophorin A is the major sialoglycopeptide of the human erythrocyte membrane (Marchesi, V. T., H. Furthmayr, and M. Tomita. 1976. The Red Cell

membrane (Marchesi, V. T., H. Furthmayr, and M. Tomita. 1976. The Red Cell Membrane. Ann. Rev. Biochem. 45:667-698 and similar O-linked sialoglycopeptides are found on the cell surface of lymphoid cells (Fukuda, M., and S. R. Carlsson. 1986. Leukosialin, A Major Sialoglycoprotein on Human Leukocytes as Differentiation Antigens, Med. Biol. 64:335-343, Remold-O-Donnell, E., A. E. Davis III, D. Kenney, K. R. Bhaskar, and F. S. Rosen. 1986. Purification and Chemical Composition of gpL115, The Human Lymphocyte Surface Sialoglycophorin that is Defective in WiskottAldrich Syndrome. J. Biol. Chem. 261:7526-7530. When glycophorin A was extensively desialated by treatment with Clostridium perfringens neuraminidase, as monitored by Warren's assay (Warren, L. 1959. The Thiobarbituric acid assay of sialic acids. J. Biol. Chem. 234:1971-1975), little or no hydrolysis of the desialated dimeric or monomeric glycophorin, by the *P. haemolytica* protease, could be seen (lane d of FIG. 9(A)). The hydrolysis of glycophorin A can be inhibition by the presence of 100 mM EDTA, but this inhibition by high concentration of EDTA was removed by dialysis of the EDTA-inhibited enzyme (lane f FIG. 9(A)). FIG. 9(B) shows that if the incubation of the glycophorin A with *P. haemolytica* pH 4.5 fractions was prolonged, then total hydrolysis of the two forms of glycophorin A was observed.

ORPL:

Neuraminidase Activity of *Pasteurella haemolytica* Isolates, Frank et al, Infection and Immunity, vol. 32 (1987) pp. 1119-1122.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KMC	Image
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2. Document ID: US 4906571 A

Entry 2 of 2

File: USPT

Mar 6, 1990

DOCUMENT-IDENTIFIER: US 4906571 A

TITLE: Cell surface modification using a novel glycoproteinase of *Pasteurella haemolytica*

BSPR:

It is also known that a non-protease, neuraminidase is widely distributed in a variety of organisms, such as myxoviruses bacteria and animals. Recently, neuraminidase activity was detected in the culture fluid of *Pasteurella haemolytica*, Otulakowsky, G. L. et al, Infect. Immun., 42, 64-70 (1983). Neuraminidase from these various sources including *Pasteurella haemolytica* hydrolyzes terminal sialic acids from glycoprotein and ganglioside oligosaccharides. Neuraminidase in cleaving such carbohydrates from glycoproteins does not in any way cleave the protein at any amino acid sites. However, Otulakowsky et al (supra) discovered that the supernatant from the culture of *Pasteurella haemolytica* not only had neuraminidase activity, but also had proteolytic enzyme activity in releasing sialo glycopeptides from glycoprotein. It was uncertain as to whether or not the enzyme activity was due to a new enzyme or previously known enzymes.

DEPR:

The glycoprotease activity is associated with a large molecular weight complex which is isolated from the culture of *Pasteurella haemolytica*. The complex is made up of at least five polypeptide chains, one or more of which is the glycoprotease. The polypeptides in the complex are tightly associated. It has been established that the polypeptides are not covalently bound, but are held together by highly stable non-covalent interactions. In addition to the glycoprotease activity of the complex, neuraminidase activity is also found in the complex. By precipitation and isolation of the complex from the culture of *Pasteurella haemolytica*, the enzymes have been purified to specific activities about seventy fold higher than that of the crude enzyme in the culture media, where both enzymes are associated with the complex. The complex is readily soluble in water so that the glycoprotease, even in its unisolated form in the complex, is readily usable in water solutions of concentrations of 70 mg of the complex per ml of water. The lyophilized enzyme complex can be stored at room temperature for at least two months without significant loss in activity, so that the glycoprotease, even in the complex form, is readily usable for cleaving the protein portion having the carbohydrate antigen markers from glycoproteins.

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